

Appl. No. 09/823,649
Amdt. dated May 16, 2006
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1634

PATENT

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-12. (Cancelled)

13. (Previously Presented) A method for reverse transcribing an RNA, that comprises:

(a) providing a reverse transcription reaction mixture comprising said RNA, a primer, Mg²⁺, and a mutant thermoactive DNA polymerase, wherein said mutant DNA polymerase is characterized in that

i) in its native form said DNA polymerase comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:1;

ii) the amino acid at position 2 of said amino acid sequence is S or A and the amino acid at position 5 of said amino acid sequence is L or I; and

iii) the amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, or P; and

(b) treating said reaction mixture at a temperature sufficient for said mutant DNA polymerase to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA.

14. (Previously presented) The method of Claim 13, wherein said mutant DNA polymerase in its native form comprises an amino acid sequence that is SEQ ID NO:2, the amino acid at position 3 of said amino acid sequence is Q or G, and the amino acid at position 6 of said amino acid sequence is S or A.

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15. (Previously presented) The method of Claim 13, wherein said mutant DNA polymerase in its native form comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:3.

16. (Previously presented) The method of Claim 13, wherein said mutant DNA polymerase in its native form comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:4, and the amino acid at position 3 is Q or G.

17-19. (Cancelled)

20. (Original) The method of Claim 13, wherein said mutant DNA polymerase is thermostable.

21. (Previously presented) The method of Claim 13, wherein said mutant DNA polymerase is a mutant form of a *Thermus* species DNA polymerase.

22. (Previously presented) The method of Claim 13, wherein said mutant DNA polymerase is a mutant form of *Thermus thermophilus* DNA polymerase or *Thermus aquaticus* DNA polymerase.

23. (Original) The method of Claim 13, wherein said temperature of said reaction mixture in step (b) is between 40°C and 80°C.

24. (Previously presented) The method of Claim 13, wherein said amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, P, Q, or D.

25-26. (Cancelled)

27. (Original) A method for amplifying an RNA, that comprise:
(a) reverse transcribing said RNA according to a method of Claim 13 to provide a cDNA;

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(b) amplifying said cDNA.

28. (Original) A method of Claim 27, wherein in step (b) said amplifying is carried out using a polymerase chain reaction.

29. (Previously presented) A method for amplifying an RNA using a single-enzyme reverse transcription/amplification reaction, that comprises:

(a) providing an amplification reaction mixture comprising said RNA, a pair of primers, a divalent cation, and a mutant thermostable DNA polymerase, wherein said mutant DNA polymerase is characterized in that

- i) in its native form said DNA polymerase comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:1;
- ii) the amino acid at position 2 of said amino acid sequence is S or A and the amino acid at position 5 of said amino acid sequence is L or I; and
- iii) the amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, or P; and

(b) treating said reaction mixture at a temperature sufficient for said mutant DNA polymerase to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA;

(c) treating said reaction mixture at an appropriate temperature for said mutant DNA polymerase to initiate synthesis of an extension product of said second primer to provide a double-stranded cDNA molecule; and

(d) amplifying said double-stranded cDNA molecule of step (c) by a polymerase chain reaction.

30. (Previously presented) The method of Claim 29, wherein said mutant DNA polymerase in its native form comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:2, the amino acid at position 3 of said amino acid sequence is Q or G, and the amino acid at position 6 of said amino acid sequence is S or A.

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31. (Previously presented) The method of Claim 29, wherein said mutant DNA polymerase in its native form comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:3.

32. (Previously presented) The method of Claim 29, wherein said mutant DNA polymerase in its native form comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:4, and the amino acid at position 3 is Q or G.

33-35. (Cancelled)

36. (Original) The method of Claim 29, wherein said mutant DNA polymerase is thermostable.

37. (Previously presented) The method of Claim 29, wherein said mutant DNA polymerase is a mutant form of a *Thermus* species DNA polymerase.

38. (Previously presented) The method of Claim 29, wherein said mutant DNA polymerase is a mutant form of *Thermus thermophilus* DNA polymerase or *Thermus aquaticus* DNA polymerase.

39. (Original) The method of Claim 29, wherein said temperature of said reaction mixture in step(b) is between 40°C and 80°C.

40. (Original) The method of Claim 29, wherein said amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, P, Q, or D.

41. (Previously presented) A method for amplifying an RNA using a single-enzyme reverse transcription/amplification reaction, that comprises:

(a) providing an amplification reaction mixture comprising said RNA, a pair of primers, Mg²⁺, and a mutant thermostable DNA polymerase, wherein said mutant DNA polymerase is characterized in that

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- i) in its native form said DNA polymerase comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO: 1;
- ii) the amino acid at position 2 of said amino acid sequence is S or A and the amino acid at position 5 of said amino acid sequence is L or I; and
- iii) the amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, or P; and

(b) treating said reaction mixture at a temperature sufficient for said mutant DNA polymerase to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA;

(c) treating said reaction mixture at an appropriate temperature for said mutant DNA polymerase to initiate synthesis of an extension product of said second primer to provide a double-stranded cDNA molecule; and

(d) amplifying said double-stranded cDNA molecule of step (c) by a polymerase chain reaction.

42. (Previously presented) The method of Claim 41, wherein said mutant DNA polymerase in its native form comprises an amino acid sequence that is SEQ ID NO:2, the amino acid at position 3 of said amino acid sequence is Q or G, and the amino acid at position 6 of said amino acid sequence is S or A.

43. (Previously presented) The method of Claim 41, wherein said mutant DNA polymerase in its native form comprises a polymerase domain comprising an amino acid sequence that is SEQ ID NO:3.

44. (Previously presented) The method of Claim 41, wherein said mutant DNA polymerase in its native form comprises an amino acid sequence that is SEQ ID NO:4, and the amino acid at position 3 is Q or G.

45-47. (Cancelled)

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48. (Original) The method of Claim 41, wherein said mutant DNA polymerase is thermostable.

49. (Previously presented) The method of Claim 41, wherein said mutant DNA polymerase is a mutant form of a *Thermus* species DNA polymerase.

50. (Previously presented) The method of Claim 41, wherein said mutant DNA polymerase is a mutant form of *Thermus thermophilus* DNA polymerase or *Thermus aquaticus* DNA polymerase.

51. (Original) The method of Claim 41, wherein said temperature of said reaction mixture in step (b) is between 40°C and 80°C.

52. (Original) The method of Claim 41, wherein said amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, F, Q or D.

53-68. (Cancelled)